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INTERACTIONS BETWEEN LIGHT AND CIRCADIAN RHYTHMS IN PLANT PHOTOPERIODISM*†

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Abstract—The timing mechanism in plant photoperiodism seems to involve two endogenous circadian rhythms: a light-on (dawn) rhythm and a light-off (dusk) rhythm. Following a period of darkness light may affect flowering without affecting the rhythms, or it may affect flowering by rephasing the rhythms. A hypothesis is presented concerning the mechanism of the interaction between illumination and endogenous rhythms based upon correlations between leaf movements and flowering responses in various photoperiodic treatments. The possible role played by phytochrome in the response is considered in relation to the effects of light quality on the responses.

BÜNNING hypothesized that the photoperiodic responses of plants are based on rhythmic endogenous changes with peaks occurring approximately every 24 hr, and that there are two 12-hr phases which are differentially sensitive to light[1, 2]. In our interpretation, we postulate that flowering is suppressed in a short-day plant and enhanced in a long-day plant when light is given during the second phase. The effect of light on flowering generally has been studied either with light perturbations during long dark periods or with variable cycle length experiments. In 1955 Blaney and Hamner[3] and later Nanda and Hamner[4] using variable cycle length treatments, clearly demonstrated the participation of a circadian rhythm in the flowering of Biloxi soybean, *Glycine max*, a short-day plant. Soybean plants given seven cycles consisting of 8 hr of light and various lengths of darkness had a rhythmical flowering response with peaks of flowering occurring in cycle lengths which were multiples of 24 hr (Fig. 1). Similar flowering rhythms were also found later in the short-day plants, *Pharbitis nil*[5] and *Chenopodium rubrum*[6].

The majority of the evidence for the involvement of circadian rhythms in flowering has been based on work with short-day plants. Recently Hsu and Hamner[7] found a circadian rhythm in the flowering of a long-day plant, *Hyoscyamus niger*. *Hyoscyamus* plants were given repeated cycles consisting of 6 hr of light and various lengths of darkness. Plants given 12-, 36-, or 60-hr cycles flowered earlier than those plants which received 24-, 48-, and 72-hr cycles. Thus in the long-day plant *Hyoscyamus*, flowering was enhanced when given cycle lengths that were *not* multiples of 24 hr. These and results from other treatments indicate that a circadian rhythm is involved in the flowering of the long-day plant, *Hyoscyamus niger*.

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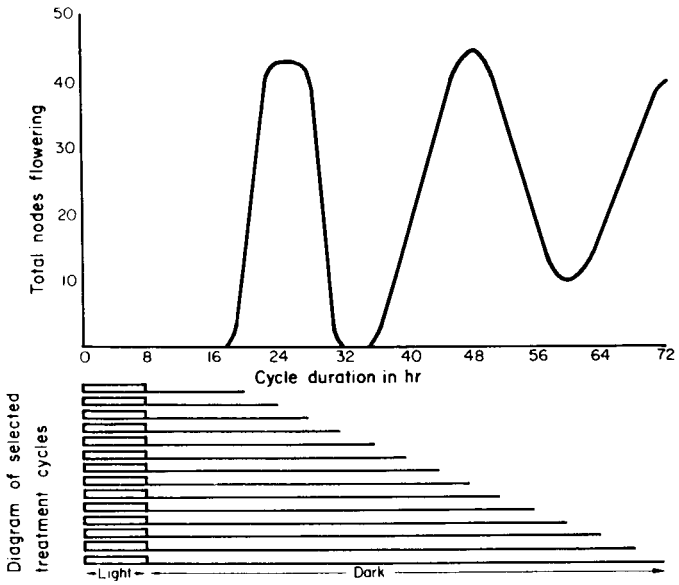


Fig. 1. Summary response curve for Biloxi soybean of six representative experiments. Plants were exposed to seven cycles, each cycle consisting of 8 hr of high-intensity light (1000–1500 ft-c) and associated dark periods of various lengths. One cycle for a few selected treatments are diagrammed below the graph for illustration. Total nodes flowering per ten plants is plotted against cycle length. The standard error for high and low points of the curve was calculated. Standard error for flowering response at cycle durations of 24, 48, 60, and 72 hr was 0·15, 0·17, 0·45, and 0·25, respectively [24].

TIMING MECHANISMS

In recent years evidence has accumulated to indicate that there may be more than one timing mechanism involved in the flowering of plants. This was shown by Takimoto and Hamner with the short-day plant *Pharbitis nil* [5], where at least three components have been found. The first is an hour glass type, and the other two are rhythmical in nature and have a period length of approximately 24 hr (Fig. 6).

Hour glass component

Evidence supporting the hour glass hypothesis was found in the following type of experiment. *Pharbitis nil* plants were germinated and were grown for 4 days in continuous light. At the end of the 4th day they were given dark periods of various lengths, and then the plants were placed in a greenhouse to develop under non-inductive conditions. With these treatments, flowering increased as the dark period was lengthened. The hour glass component was also found to be affected by temperature with a greater number of flowers developing when the temperature is higher.

Light-on rhythm

If the proper light-dark treatment is given prior to the main dark period, one of the rhythmical components, the light-on rhythm, is initiated by the onset of the light

period. *Pharbitis* plants were grown for 4 days in continuous light and then given a non-inductive treatment of 8 hr dark and 12 hr light or 8 hr dark and 8 hr light. Dark periods of various lengths were then given followed by long days in the greenhouse. The flowering response curve of plants given a 12 hr light period was found to coincide with the curve of plants given 8 hr of light when the curves were plotted in relation to the beginning of the light period (Fig. 2). These results indicated that the flowering response was directly associated with the beginning of the light period, with maximum floral inhibition being 20 hr after the beginning of the light period. Results from similar experiments on *Xanthium* by Moore *et al.*[8] concur with the *Pharbitis* results and suggest that a light-on response may also occur in the flowering of *Xanthium*. The data of Papenfuss and Salisbury[9] indicate that maximal floral inhibition in *Xanthium* is about 14 hr after the beginning of the light period instead of 20 hr. Evidence for the possible participation of a light-on rhythm was also found in *Biloxi* soybean by Shumate *et al.*[10].

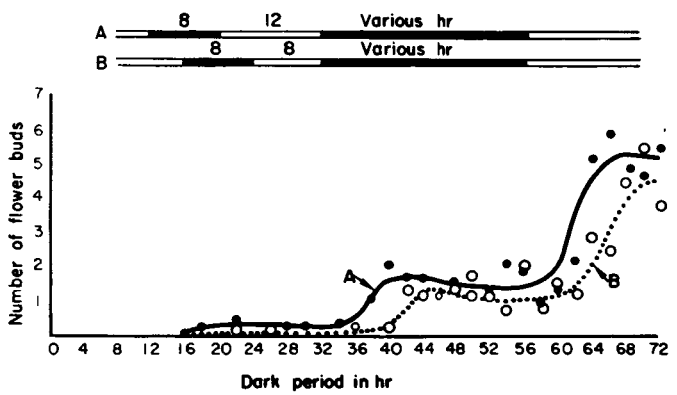


Fig. 2. Flowering response of *Pharbitis nil* at 18°, exposed to a single dark period of various durations preceded by different light conditions. Light conditions preceding the main dark period are shown diagrammatically[5].

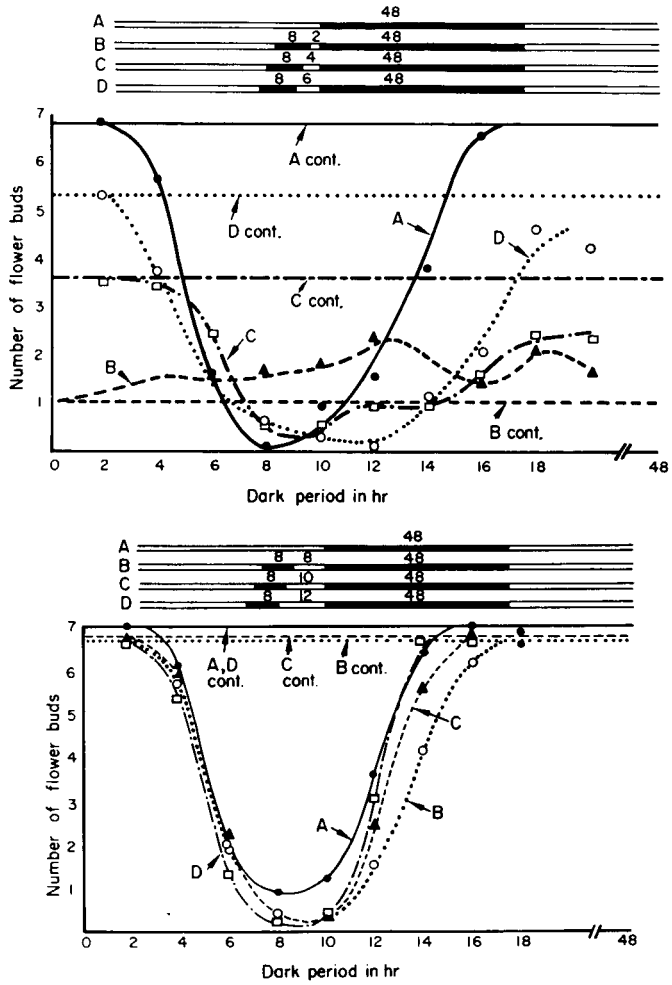
Light-off rhythm

A rhythmical component initiated by the beginning of the dark period (light-off rhythm) was demonstrated in experiments where the main dark period was interrupted with red light*[5]. *Pharbitis nil* plants were grown for 4 days in continuous light and then given a 48-hr dark period. This dark period was interrupted at various times by 5 min of red light. Maximum floral inhibition occurred when red light was given at the 8th hour of darkness. A second inhibition though not as pronounced, occurred 24 hr later when light was given at the 32nd hour of darkness. In *Xanthium*, given 24, 48 or 72 hr dark periods, maximum floral inhibition by red light interruptions also occurred 8 hr after the beginning of darkness[8, 9]. These results may indicate the presence of a light-off rhythm in *Xanthium*.

By introducing a non-inductive photoperiodic treatment prior to the long dark

*In experiments of this nature, the red light used has a peak emission at 660 nm, and approximately 95 per cent of the light energy is within 600 to 700 nm.

period, the participation of this rhythmic dark-initiated component was clearly demonstrated in *Pharbitis nil*[5]. The degree of floral inhibition by red light interruptions at the 8th hour of the main dark period was dependent on the length of the light period preceding the main dark period. An inhibitory effect was not seen when the length of the preceding light period (4000 lx) was less than 4 hr. But as the length of the preceding light period was increased from 4 to 12 hr there was concomittent increase of floral inhibition due to the light-off rhythm. Two figures of the actual flowering response under these experimental conditions are presented (Figs. 3, 4), and in the third figure the theoretical curves are presented (Fig. 5). Thus the light-off rhythm appears to require at least 4 hr of light for initiation. Longer light periods of up to 12 hr appear to intensify this rhythm.



Figs. 3, 4. Flowering responses of *Pharbitis nil* to red light interruptions given at different times in the first half of a 48-hr dark period which was preceded by various light conditions. The light conditions preceding the 48-hr dark period are shown diagrammatically. Dark-period temperatures were 18·5° in Fig. 3 and 19° in Fig. 4[5].

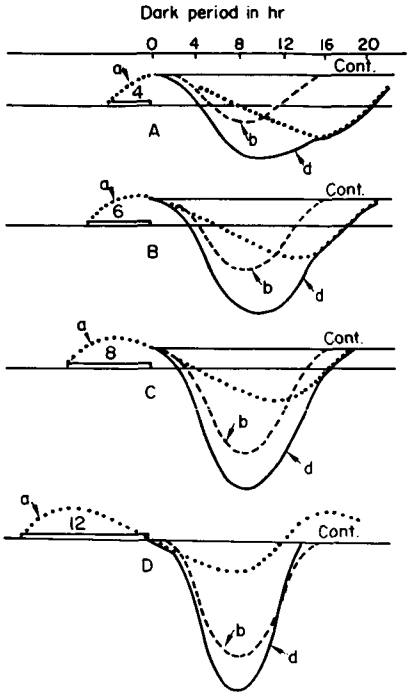


Fig. 5. Theoretical curves for flowering responses of *Pharbitis nil* exposed to 5 min of red light at different times in a 48-hr dark period [5].

Interactions of light-on and light-off rhythms

Further qualities of the light-on and light-off rhythms were discovered in experiments with *Pharbitis nil* where pretreatments of 8 hr of dark and 8 hr of low intensity light were given prior to a 48-hr dark period which was interrupted at various times by red light [11]. When an intensity of 10 lx from fluorescent lamps was given during the pretreatment, the light can initiate a light-on rhythm but apparently can not initiate a light-off rhythm. On the other hand 8 hr of 70 lx light can initiate a light-off rhythm. In contrast to this, two hours of 4000 lx of light preceding the main dark period were not sufficient to start a new rhythm [5]. Thus it appears that the initiation of the light-on rhythm can be accomplished by light per se (10 lx) while on the other hand the initiation or the re-initiation of the light-off rhythm may be influenced more by the duration of the illumination rather than by the total amount of the light received.

Further evidence concerning light-on and light-off rhythms was found in the petal movement of *Kalanchoe* flowers. In these experiments by Engelmann [12], the plants were exposed to either a long period of continuous light followed by continuous darkness or vice versa and the resulting petal movements were observed. During the continuous light period a loss of petal movement occurred. When the light was then turned off, a rhythmic movement of the petals was initiated with the first closure of the flower petals occurring about 5 hr after the beginning of the dark period. When the plants were given a long dark period a loss of petal movement also occurred. When

the light was then turned on, the first closure of the flower occurred about 15 hr after the beginning of the light period. Engelmann pointed out that the time of maximum petal closure in both the light-on and the light-off rhythms would coincide if a plant left in darkness is given 10 hr of light. He suggested in a later paper[13] that an enhancement of petal movement would occur from the interaction of the light-on and the light-off rhythms if 9 hr of light is given prior to the main dark period. This 9 hr length of the light period also happens to be optimal for flower induction in *Kalanchoe*[14]. However, until more information is gathered, one can only speculate whether the coincidence of the optimal length of light period for the petal movement and for flowering is accidental or whether flowering induction is optimal when synchronization of the light-on and light-off rhythm occurs.

PHYTOCHROME

The flowering response of plants to red and far-red light* has been previously considered to be strictly a response mediated by phytochrome. However this may not be the case, since the red and far-red response has been shown to be highly dependent not only on when the light treatment is given during the dark period[15, 16] but also on the length of the dark period given to the plant. With short dark periods, far-red light reverses the inhibition produced by red light, whereas under longer dark periods far-red is inhibitory and in fact additive to the red inhibitory effect (Table 1). The red and far-red interaction on flowering may be better understood if the results of Takimoto and Hamner are considered[17].

Table 1. Response of *Xanthium* to various lengths of darkness interrupted by 5 min of red (R) and/or far-red (FR) light. A dark period (DP) of 12, 16 or 48 hr was given. Light interruptions were given at the 6th, 8th or 8th hours of the dark period respectively. Data were compiled from 3 tables [15]

Treatment	Mean flowering stage		
	12 hr DP	16 hr DP	48 hr DP
Dark control	3·3	3·9	6·0
R	0·0	0·2	4·3
R + FR	2·2	0·0	0·3
FR	3·8	2·7	2·9
FR + R	0·0		4·4

Pharbitis seedlings raised as previously described were exposed to 5 min of far-red light at different times during the 48-hr experimental dark period. The flowering response curve followed closely that of the hour glass component and did not show a rhythmic response. However, when far-red light was followed immediately by red, the flowering response was as though only red light was given. If the sequence was reversed, red followed by far-red, the effect of the two lights appeared to be additive. A similar type of response was also observed in experiments where red and far-red were given simultaneously in varying ratios. A contamination of red light

*In experiments of this nature, the far-red light emission is greater than 710 nm. See Reid *et al.*[15] for emission spectra curves.

by far-red did not materially affect the red light response. On the other hand, the effect of a slight contamination of the far-red light by red was additive. Thus, once red light is given, a red light effect is present and this effect is not reversed by subsequent exposure to far-red light. When far-red is followed by red light, the far-red effect is reversed by red light. These results (non-reversal of the red effect and reversal of the far-red effect) suggest that the flower inhibiting effects of red and far-red light can be based on different mechanisms. According to Takimoto[17] red light slows down the hour glass component of the timing mechanism, and this effect is not reversed by subsequent exposure to far-red. Far-red light stops the hour glass component of the timing mechanism, and this far-red effect can be reversed by subsequent red irradiation. It was therefore suggested that some non-reversible pigment absorbing red energy is concerned with the red effect, and that the reversible pigment, phytochrome, is concerned only with the stopping of the hour glass component by far-red.

LEAF MOVEMENTS

Much of Bünning's contribution to the field of circadian rhythms during the past 40 years was from experimental data on the leaf movements of *Phaseolus multiflorus*. The effects of temperature, light, and chemicals on the leaf movements have been well documented by Bünning and his students[18]. *Phaseolus*, being a day-neutral plant flowers independently of day length. Therefore correlations of leaf movements to flowering response are difficult and mostly meaningless. One of the best studied short-day plants is *Xanthium strumarium*, and its flowering response to various light and dark treatments is well known. Furthermore, it now appears that two distinct types of rhythms are present in the leaf movements of *Xanthium*[19]. One rhythm is initiated by the beginning of a light period, and the other rhythm is initiated by the beginning of a dark period. These leaf movement rhythms have been designated the 'light-on' and 'light-off' rhythms respectively. A maximum closure (upward movement) of the leaf in red light for at least the first 24 hr of the dark period. Because of these similarities the general movement of the leaves is similar to the curve of floral inhibition by white or red light for at least the first 24 hr of the dark period. Because of these similarities the leaf movements of the 'light-off' rhythm seem to be related to the flowering response in *Xanthium*.

Recently Brest[20] has reported that light perturbation treatments may either interact with a rhythm or may phase-shift a rhythm. These interpretations are based on evidence of strong correlations between leaf movements and flowering in Biloxi soybean. The plants were given seven 72-hr cycles each consisting of 8 hr of light followed by 64 hr of darkness. To test the response of leaf movement and the flowering response of Biloxi soybean, the dark period was interrupted with 3 min, 30 min, 1 hr, 2 hr, or 4 hr of white light at the 16 hr point of the cycle where flowering response is strongly inhibited by light perturbations. The leaf movements were observed throughout the 7 cycles of treatment, and the flowering response was determined subsequently by dissection. Brest concluded that all light perturbations he used including the 3 min treatment were sufficient to completely inhibit flowering. However in the case of leaf movements, the 3 min light perturbation did not affect the leaf movement rhythm. On the other hand light perturbation of 30 min and 1 hr caused what was apparently a photonastic response in the leaf movement. A slight phase-shift of the leaf movement

rhythm was induced by a 2-hr light perturbation. A definite phase-shift of the leaf movement rhythm was induced by a 4-hr light perturbation.

These results indicate that light perturbations at the 16-hr point may have different effects on flowering and on leaf movements. If we assume that the leaf movements are indicative of the status of the underlying circadian rhythm (basic rhythm), we can hypothesize that at the 16-hr point, a short light perturbation does not affect the basic rhythm but only interacts with the flowering process since the leaf movement rhythm was not disturbed. But since the leaf movement rhythm was rephased by a 4-hr light perturbation, we can also hypothesize that at the 16-hr point, a long light perturbation (4 hr) is required to rephase or to re-initiate the basic rhythm. Thus it appears that at the 16-hr point, flowering is affected primarily by the interaction of the light and the basic rhythm, while the leaf movement is affected by rephasing the basic rhythm by a long light treatment.

In contrast to the results obtained at the 16-hr point, Brest found that at the 40-hr point, 30 min of light rephased the leaf movement rhythms. Furthermore the phase-shift observed (Fig. 6) was very similar to the phase-shift of the flowering rhythm reported by Nanda and Hamner for Biloxi soybean given comparable experimental conditions [21]. We can further hypothesize that the flowering and the leaf movement rhythms are very similar and/or are closely coupled since a 30 min light perturbation at the 40-hr point rephased both flowering and leaf movement rhythms in a nearly identical manner.

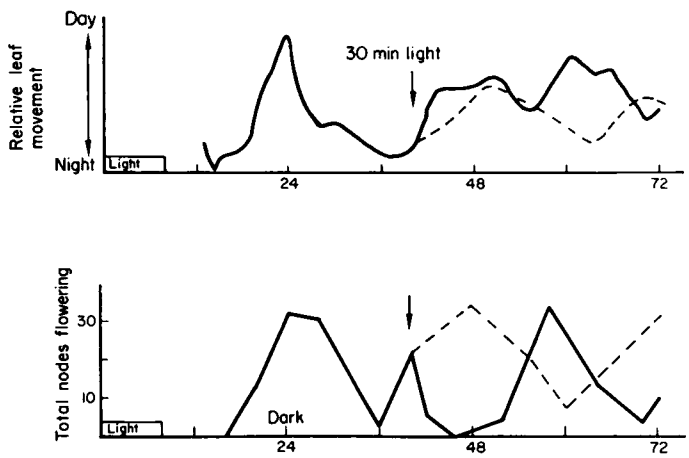


Fig. 6. (a) The effect on the leaf movement rhythm of a 30 min light perturbation given at the 40 hr point of a 72 hr cycle. The dotted line represents the control which received no perturbation. (b) The effect on the flowering rhythm of a 30 min light perturbation given at the 40 hr point. The dotted line represents the control which received no light perturbation. Data from Nanda and Hamner [4].

Thus from the evidence obtained from light perturbation treatments of Biloxi soybeans at the 16-hr or at the 40-hr points of the cycle, one must now consider whether the results of the perturbation given at various times during the rhythmic cycle were due to an interaction with the basic rhythm or were due to a phase-shift of the rhythm.

SUMMARY

Three components which participate in the flowering response of *Pharbitis* have been defined as (1) an hour glass component, (2) a light-on component, and (3) a light-off component (Fig. 7). The hour glass component is non-rhythmical, is initiated by the end of a light period, stopped and re-initiated by far-red and red light respectively, and is temperature sensitive. The rhythmical light-on component is initiated by the beginning of the light period, requires only 10 lx of light for initiation, is affected by red and white light, and has maximum floral inhibition by light 20 hr after the beginning of a light period. The light-off component which is also rhythmical, is initiated by the end of a light period which must be at least 70 lx intensity and of several hours duration, has maximum floral inhibition at the 8-hr point and is temperature insensitive.

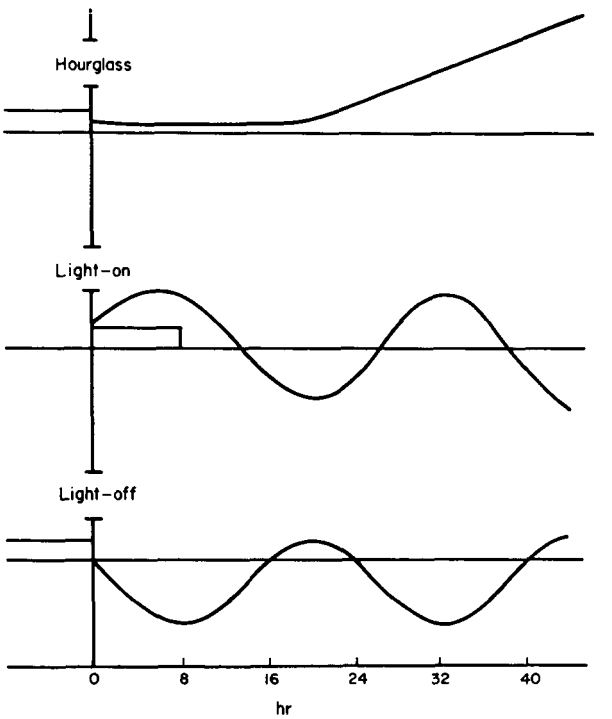


Fig. 7. Theoretical curves of the three components which participate in the flowering of *Pharbitis nil*.

Additional evidence indicating the participation of one or more of these components has been reported for the flowering response of *Xanthium* and Biloxi soybeans, for the petal movement of *Kalanchoe*, and for the leaf movements of *Xanthium*.

From recent investigations, it has been hypothesized that the effect of light perturbation on the flowering of Biloxi soybean during dark periods may be due to either an interaction between the light and the basic rhythm or may be due to a rephasing of the basic rhythm. The response elicited is apparently dependent on the status of the basic rhythm when the light perturbation is given and also on the length of the light perturba-

tion. Furthermore, evidence of a correlation between the leaf movement rhythm and the flowering rhythm has also been reported in Biloxi soybean.

Bünning's hypothesis has provided the impetus for vast amounts of research concerning the flowering response of plants. It is now well established that a rhythmic process participates in the flowering of plants. Evidence is also in hand indicating the presence of more than one timing mechanism. Some of the characteristics of these mechanisms are now being understood. The evidence for the participation of circadian rhythms has been, by and large, from flowering and from leaf or petal movement data. Although a periodic physico-chemical reaction of high frequency has been reported [22] and circadian molecular models have been suggested[23], very little direct evidence for a 'molecular mechanism' concerned with circadian rhythms has been presented.

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